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Adhesion and Proteome Analyses of Lactobacillus acidophilus NCFM grown with the potential Prebiotic Raffinose

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CONFERENCE PROCEEDINGS

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11th - 13th June 2013
DoubleTree by Hilton
Kosice, Slovakia

www.probiotic-conference.net

International Scientific Conference on Probiotics and Prebiotics – IPC2013
June 12, 2013 - DAY 2

9:00 SESSION 5; Chair: E. McCartney, Co-Chair: S. Christiaen

KEYNOTE Lecture
E. McCartney: What's New (& Old!) in the Regulation of EU Probiotics?

M. M. Sanad: Resolution of Cryptosporidiosis by Probiotics: A True Medical Breakthrough or Just a Fervent Hope?

A. Aleshkin: Phage-Based Probiotic Dietary Supplement in Prophylaxis against Food-Borne Infections

E. Dalzini: Lactic Acid Bacteria and Foodborne Pathogens: Predictive Models Applied to an Italian Goat Cheese

S. Christiaen: The Interspecies Signaling Molecule Autoinducer-2 Plays an Important Role in the Probiotic Effect of Bifidobacteria

10:55 Coffee Break

11:15 SESSION 6; Chair: N. Kabanova, Co-Chair: E.C.P. De Martinis

KEYNOTE Lecture
N. Kabanova: Microcalorimetric Approach in Studies of Growth of Microorganism in Different (Food) Matrices

E. C. P. De Martinis: Isolation, Identification and Characterization of Antibacterial and Proteolytic Activities of Lactic Acid Bacteria Isolated from Cow, Buffalo and Goat's Milk and Cheese

H. U. Celebioglu: Adhesion and Proteome Analyses of Lactobacillus Acidophilus NCFM Grown with the Potential Prebiotic Raffinose

12:30 Lunch

13:30 SESSION 7; Chair: L. Stenman, Co-Chair: A. K. Makinen

KEYNOTE Lecture
L. Stenman: Probiotics against Metabolic Syndrome

KEYNOTE Lecture
A. K. Makinen: Probiotics for skin health

T. Satoh: Effect of Bifidobacterium breve M-16V on Necrotizing Enterocolitis in Neonate Rat Model

D. Wang: Modulation of Innate Immune Response In Vitro by a Combination of Arabinoxylan Oligosaccharides and Bifidobacterium Lactis NCC2818

Introduction

There is an interest on the use of probiotics to modulate unbalanced human and animal microbiota. Inflammatory bowel diseases (IBD) are a mayor cause of consultation and death worldwide. Advances in the pathogenesis of bowel inflammation show that probiotics could prove to be a useful treatment. This work evaluated the protective role of a preparation based on *Lactobacillus salivarius* (LPLM-O1) in a murine model of enterocolitis.

Methods

C57BL/6 mice were randomized in three groups: 1) a control group, treated with phosphate-buffered saline (PBS); 2) a group, treated with oral doses of 3% dextran sulfate sodium (DSS) in order to induce IBD, and 3) a group treated with daily oral doses of DSS and 10^8 cfu/mL of LPLM-O1 for 7 days. Lactic acid and Gram-negative bacteria, as well as immunohematologic, histologic and clinical parameters, were quantified for each group at the 7^{th} and 14^{th} days.

Results

A LAB count in the faeces and intestines of animals from all groups was 10^8 cfu/gr. For Gram-negative bacteria, there was a count of 10^6 cfu/gr in the intestines of the group treated with DSS and LPLM-O1 at day 7, and then it fell to 10^4 cfu/gr in day 14. At the third day of administrating 3% DSS, an increase in the concentrations of eosinophils, neutrophils, lymphocytes, and monocytes –when compared to the PBS group ($p<0.05$)– could be observed; this lasted 14 days, without seeing a normalization during the whole experiment. IL-6 levels in the spleen increased in the DSS group and diminished in the LPLM-O1 group ($p<0.05$), in contrast with what happened in the mesenteric lymph nodes, where a higher concentration of IL-6 was observed for group 3 ($p<0.05$) at day 14. Histologically, a remarkable recovery was observed in the animals treated with DSS and LMLM-O1; when compared with those treated only with DSS, the damage decreased on 40% in the duodenum, 10% in the jejunum, 50% in the ileum, and 40% in the colon. These results match what was clinically observed.

Conclusion

The inclusion of 3% DSS in the diet of C57BL/6 mice generated an acute alteration on the bowel tissue, with severe damage. Animals treated with DSS and *Lactobacillus salivarius* showed a substantial improvement in their condition.

Research financed by INNOVA CHILE project 09CAVC-6955.

Keywords: probiotic , Inflammatory bowel diseases, lactobacilli, enterocolitis, Lactobacillus salivarius

Adhesion and Proteome Analyses Of Lactobacillus Acidophilus NCFM Grown With The Potential Prebiotic Raffinose

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Introduction

Probiotics are "live microorganisms which when administrated in adequate amounts confer a health benefit on the host" (FAO/WHO). There are certain criteria to be probiotics: resistance to and colonization of the human gastrointestinal tract (GIT), antimicrobial activity against potential pathogens, and modulation of the immune system. *Lactobacillus acidophilus* NCFM (NCFM) is a well studied probiotic bacterium adapted to survive in the GIT and it shows potential health benefits to the host [1].

Non-digestible oligosaccharides are energy sources for the microorganisms in the GIT. They can have important health benefits for humans by stimulating growth of beneficial bacteria; therefore, they can be classified as prebiotics [2]. Raffinose belonging to the raffinose oligosaccharide family of potential prebiotic oligosaccharides is a trisaccharide composed of galactose, fructose, and glucose and was shown to stimulate growth of *L. acidophilus* [3].

Organs exposed to the external environment secrete mucus. The viscous and elastic gel-like properties of mucus stem from mucins, which are large extracellular glycoproteins with a tendency to adhere to other

substances, including lipids [4]. The present study aims to identify changes in the proteome of NCFM and its adhesion to mucins by the utilization of raffinose.

Methods

NCFM was grown in LABSEM media supplemented with 1% glucose (as control) or 1% raffinose [1].

For proteome analysis, whole cell extracts were separated by 2D gel electrophoresis using pH 4–7 for the first dimension and SDS-PAGE in the second dimension, followed immediately by image analysis. Protein identifications of differentially abundant spots were performed by using Mass Spectrometry (MS) [1].

For assay of adhesion, NCFM grown with glucose or raffinose was incubated with mucins in polystyrene microtiter plate. Adhesion was expressed as the percentage of fluorescence recovered after binding to mucin relative to the fluorescence of the bacteria added to the wells [5].

Results and Discussion

In total 108 protein spots, 63 spots were identified by MS. Forty eight protein spots showed higher, while 15 showed lower relative abundance. The 63 identifications corresponding to 43 unique proteins are distributed among 12 functional categories. Several glycoside hydrolases were identified to be differentially abundant when NCFM was grown on raffinose as compared to glucose. These include α-galactosidase, large and small subunit of β-galactosidase, and sucrose-6-P hydrolase. Raffinose induces the NCFM raffinose operon, consisting of genes encoding an ABC transporter, α-galactosidase and sucrose phosphorylase [5].

Several differentially expressed proteins (7%) belong to the category "cell envelope" [5]. This proposes that growth on raffinose alters adhesive properties of NCFM and mucin adhesion assay showed that adhesion of NCFM was increased significantly when grown on raffinose as compared to glucose since proteins having roles in adhesion have differential expression profile due to growth on raffinose.

Acknowledgments

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Keywords: Lactobacillus acidophilus NCFM, raffinose, mucin, 2D-DIGE, adhesion

Immunomodulatory Phi FC1 Phage from Enterococcus Faecalis Activates Interleukin-12 and Nuclear Factor-κB in Dendritic Cells of Mouse Bone Marrow

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Introduction

Phages entering eukaryotic hosts are considered as a highly immunogenic foreign antigen which could interact with the innate immune system. The bacteriophage Phi FC1 was first isolated in our laboratory from a culture of lysogenic strain of Enterococcus faecalis (KBL 703) after induction by ultraviolet (UV) irradiation. In this study, we examined whether Phi FC1 phages can influence surface molecule expression, cytokine production, and their underlying signaling pathways in murine bone marrow-derived DCs. In DCs, Phi FC1 activated NF-κB p65 and triggered its nuclear translocation as determined by western blotting and immunofluorescence assay. Also, Phi FC1 significantly induced expression of the IL12 in DCs. Moreover, phi FC1 enhanced IL-12-luciferase L-12 p40 is a direct target of p65. So, p65 are possibly associated with IL12 in maturation of DCs. These results indicate that Phi FC1 may have the immunomodulatory role in maturating on the DCs.

Methods

E. faecalis KBL 703 strain, the original lysogenic strain of phage FC1 was used as the source of the bacteriophage with UV irradiation. The iDCs were treated with Phi FC1, after which the cells were harvested for Western blotting to detected JNK, p-JNK, IκB, p-IκB and β-Actin as a control. NF-κB pathway is a major signaling pathway for IL-12 in Phi FC1-stimulated DCs. A, Total p65 and nuclear p65 were detected by Western blot analysis using their specific antibodies. Cells were stained with a rabbit polyclonal Ab to p65 protein and with anti-rabbit Alexa Fluor 568-conjugated Ab. NF-kB is upstream signaling